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Tropical *Trametes lactinea* is widely distributed in the eastern USA

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ABSTRACT *Trametes lactinea* is reported from the USA for the first time. This large and conspicuous polypore, known until now only from tropical regions, was collected in many different localities in eastern USA. ITS region of six collected specimens and four others from public and private herbaria was sequenced and compared with the sequences in the GenBank. Important macro- and micro-characteristics that discriminate between *T. lactinea* and its similar species *T. elegans* are pointed out and discussed.

KEY WORDS Basidiomycota, USA new record, taxonomy, invasive species

Introduction

Since 2001, we have occasionally collected in the eastern USA (from Virginia to Connecticut) a very large (≤ 7 cm thick, ≤ 20 cm wide), white to light greyish *Trametes* species with a velutinous but not hirsute pileus and large, regular pores that appear a bit brownish when old. The collections, which were always sterile, could not be identified using the keys for USA *Trametes* species. In early spring 2009 when travelling around southern Florida we noted a great abundance of a very similar, but thinner and fertile, *Trametes* sp. that was in many localities the dominant fungus on both hardwoods and pines. In this article we present evidence that both the early spring/southern and the autumn/northern USA collections represent the same species, *Trametes lactinea*, that is, accordingly, widely distributed in the eastern USA and locally very common, in contrast to literature data.

Trametes lactinea is a typical *Trametes* species with a white tough context, white tomentose pileus, and large pores. Described from Sri Lanka (Berkeley 1843), it was reported as common in the tropics of East Asia and Australia, rare

TABLE 1. *Trametes lactinea* and *T. elegans* specimens examined.

SPECIMEN	*	LOCALITY	SUBSTRATE	COLLECTED
<i>T. lactinea</i>				
PRM560783	1	Fungi of west Pakistan 15286, coll. S. Ahmad	<i>Morus alba</i>	27.II.1961
Damm 8936	2	Mahé, Beau Vallon, Seychelles	<i>Cocos</i>	10.II.2008
Damm 4703	3	Mauritius Island	hardwood	13.II.2000
JV0108/8 H		Valley Forge, PA, USA	<i>Populus</i>	1.VIII.2001
JV0309/130 J		Hatfield Apartments, Hatfield, PA, USA	<i>Carya</i>	18.IX.2003
JV0309/186A	4	Promised Land St. Park, Pike County, PA, USA	beech	22.IX.2003
JV0312/28.2 J		Tree Tops Park, Miami, FL, USA	<i>Casuarina</i>	28.XII.2003
JV0410/11 J	5	Mason Neck St. Park, Woodbridge, VA, USA	<i>Nyssa</i>	9.X.2004
JV0709/179		Mason Neck St. Park, Woodbridge, VA, USA	hardwood	22.IX.2007
JV0808/29	6	Swamp Creek, Schwenksville, PA, USA	oak	28.VIII.2008
JV0809/50	7	Yale Univ. arboretum, New Haven, CN, USA	beech	13.IX.2008
JV0904/23		Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/24	8	Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/27		Royal Palm, Everglades N.P., FL, USA	hardwood	19.IV.2009
JV0904/43	9	Long Pine Key, Everglades N.P., FL, USA	pine	19.IV.2009
PRM915660		Long Pine Key, Everglades N.P., FL, USA	pine	21.IV.2009
JV0904/79		Long Pine Key, Everglades N.P., FL, USA	pine	22.IV.2009
JV0904/93		Long Pine Key, Everglades N.P., FL, USA	pine	22.IV.2009
JV0904/120		East Main Tram, Fakahatchee Strand Preserve, FL, USA	hardwood	22.IV.2009
JV0904/127		Collier Seminole St. Park, FL, USA	pine	23.IV.2009
JV0409/145	10	Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
PRM915661		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/146		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/147		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/148		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
JV0904/148A		Myakka River St. Park, Sarasota Co., FL, USA	live oak	24.IV.2009
<i>T. elegans</i>				
PRM871961		Esmeralda pr. Camagüey, Mt. California, Cuba	<i>Eugenia</i>	14.X.1966
PRM887367	11	Pico Perillo, Sierra del Escambray, Cuba Centr.	hardwood	5.I.1967
6069 Dammrich	12	Ko Samui, Thailand	hardwood	15.II.2002
JV0212/37 J	13	Gainesville, FL, USA	<i>Carya</i>	20.XII.2002
JV0411/2 Ja&Ro		Cameroon Highlands, Malaysia	hardwood	27.XI.2004
JV0511/2 Kop		Ecuador	hardwood	3.XI.2005

* Numbers indicate sequenced specimens. Bold numbers indicate specimens with ribosomal RNA ITS region sequence deposited in GenBank: **2** HM756192, **3** HM756193, **10** HM756191, **11** HM756196, **12** HM756194, **13** HM756195. Sequences 1, 4, 6, 7, 8 are identical with published sequence AY684178; sequences 5, 9, 10 are also identical.

in southern China and Africa, but not occurring in tropical America (Corner 1989, Dai 2009, Dai et al. 2004, Ryvar den 1976, Ryvar den & Johansen 1980). Since 2000, its occurrence in South America has slowly been acknowledged (Ryvar den 2000, 2001), but there are no previous records for the USA. We have compared our USA collections with three standard *T. lactinea* samples from public and private herbaria, including ITS region sequencing, and found that they are identical. We also show that other sequences in the GenBank annotated as *T. lactinea* are often derived from another species, *Trametes elegans* (Spreng.) Fr., and so we summarize here the most important characteristics that allow to discriminate these two species. Comments regarding other lenzito id *Trametes* species are also provided.

Materials & methods

Specimens studied

We collected 13 fertile specimens of *T. lactinea* in early spring 2009 in Florida and nine other sterile specimens from various states of eastern USA from late summer through early winter during 2001, 2003, 2004, 2007, and 2008. We also studied one sample (unfortunately sterile) of *T. lactinea* obtained from PRM collection in Prague and one fertile sample from Frank Dammrich private collection that was reputedly determined by Prof. Ryvar den. We have also inspected some typical samples of similar *T. elegans* (TABLE 1) and one aberrant sample from the Dammrich collection labeled as *T. menziesii* (Berk.) Ryvar den. Pieces of dried basidiocarps mounted in water were examined microscopically and 20 basidiospores from each specimen were measured. One drop of 10% KOH was added to study the swelling of skeletal hyphae. Specimens are deposited in the private herbarium of the first author (<http://mykoweb.prf.jcu.cz/polypores>) and PRM (TABLE 1).

ITS amplification and sequencing

0.25 g of the context tissue from dried specimens was frozen and disintegrated 60 s with steel ball in mixer mill MM301 RETSCH under liquid nitrogen. DNA was isolated using CTAB/NaCl extraction buffer as described by Murray & Thompson (1980), followed by repeated extraction with chloroform and isopropanol precipitation. Crude DNA was dissolved in 100 µl of sterile water and further purified using Wizard Clean Up kit PROMEGA. Resulting DNA solution (50 µl) was diluted ten times and 1 µl was used as template for amplification with ITS1 and ITS4 primers (White et al. 1990) in 25 µl reaction mixture using 55°C annealing temperature. Amplified DNA was purified using Wizard Clean Up kit PROMEGA and sequenced in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on ABI 3730xl DNA analyzer, using BigDye Terminator 3.1 kit.

ITS sequence analysis

The sequences were aligned by Clustal X and manually pruned. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). All positions containing gaps and missing data were

eliminated from the dataset (complete deletion option). There were a total of 454 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Taxonomy

Ryvarden & Johansen (1980) and Corner (1989) published very good descriptions of *Trametes lactinea*; here we compile the most important features and add some new characteristics based on our own observations of many fresh USA specimens.

Trametes lactinea (Berk.) Sacc.

FIGURE 1

Photos in (<http://mykoweb.prf.jcu.cz/polypores>)

BASIDIOMES annual to biennial, solitary to more rarely imbricate (when growing on short stumps), pileate, broadly to narrowly attached, 1–25 cm broad and wide, and 0.5–2 cm thick (exceptionally up to 7 cm thick at the base in specimens from the Northern USA), hard corky; **PILEUS** semicircular, mostly applanate but sometimes rather thick at the base, surface velvety, but with no hairs visible under 20× lens, becoming warted with age, sometimes with irregular outgrowths especially near the base, azonate or very slightly concentrically sulcate, sometimes with narrow greyish zones near the margin or with wide, concentric furrows that mark periods of an intensive growth; **MARGIN** obtuse and thick at first, sterile below, later sharpened, with pores developed to the very edge; **PORES** at first white, later cream to ochraceous and darker than the upper surface, at first round, thick-walled, later with wedge-narrowed edges, in old specimens thin-walled, distinctly angular, but never labyrinthine, 2–3 per mm; **TUBES** concolorous with the context, 6–12 mm long; **CONTEXT** 5–20 mm, sometimes up to 60 mm thick, white, corky.

Hyphal system trimitic, **GENERATIVE HYPHAE** clamped and thin-walled, 1–3 µm in diam.; **SKELETAL HYPHAE** abundant, hyaline, mostly with only moderately thickened walls, 4–6 µm in diam., but sometimes 3–4 µm broad and almost solid. After adding 10% KOH the solid hyphae swell to 10–15 µm in diam. and shrink quickly in vivid movements; in moderately thickened hyphae the swelled walls only fill the hole but the outer diameter remains unchanged. **BINDING HYPHAE** abundant, hyaline, thick-walled, arboriform, 1–5 µm in diam.; **BASIDIA** clavate, 4-sterigmate, 12–16 × 5–7 µm; **CYSTIDIA** none; **BASIDIOSPORES** oblong-ellipsoid to cylindrical, hyaline, negative in Melzer's reagent, 5–7 × 2.5–3.2 µm, thin-walled.

Differentiation between *Trametes lactinea* and *Trametes elegans*

Because *Trametes lactinea* keys in the American literature as *T. elegans* (Gilbertson & Ryvarden 1987), we summarize here the most important characteristics of both species. Spores are clearly diagnostic — they are distinctly

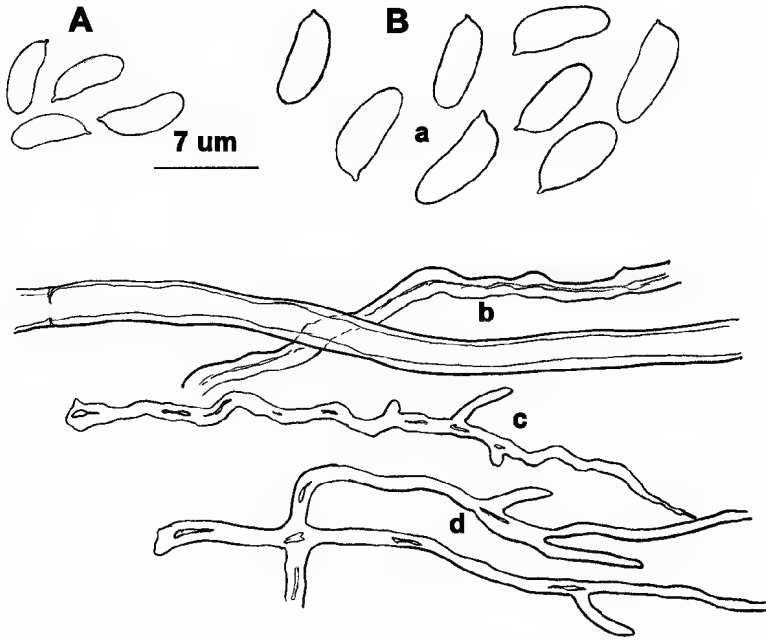


FIGURE 1: *Trametes elegans* PRM 887367 (A): basidiospores. *Trametes lactinea* (B): a. basidiospores, b. skeletal hyphae, c, d. binding hyphae.

larger in *T. lactinea* (usually $> 6 \mu\text{m} \times 3 \mu\text{m}$) and more curved and smaller ($< 6 \mu\text{m} \times 2.5 \mu\text{m}$) in *T. elegans* (FIG. 1). Basidia of *T. elegans* are also quite small, only $8\text{--}13 \times 4\text{--}6 \mu\text{m}$. Unfortunately, many collections of both species are completely sterile. *Trametes lactinea* does not have hyphal pegs but all 6 studied specimens of *T. elegans* had pegs in the tubes that could be best seen on cut tubes under lens as scattered warts or protuberances on tube sides. Although this feature has never been mentioned in *T. elegans* descriptions, we regard it as rather constant. Pores of *T. lactinea* change in the course of basidiocarp development from thick-walled, round, and white to relatively thin-walled, angular, and brownish, but they are never labyrinthine, whereas in *T. elegans* they are mostly lamellate and when poroid, they are a bit elongated or labyrinthine at least in part of the fruitbody. The KOH reaction is only moderate in *T. elegans*: the hyphae swell slightly, without shrinking accompanied by quick hyphal movements. Unfortunately, the typical swelling reaction of *T. lactinea* skeletal hyphae is much weaker or less striking in very old herbarium specimens. The pileus surface of *T. lactinea* is quite variable but never glabrous, as it appears in most specimens of *T. elegans* (and never hirsute like by *T. gibbosa* (Pers.) Fr.).

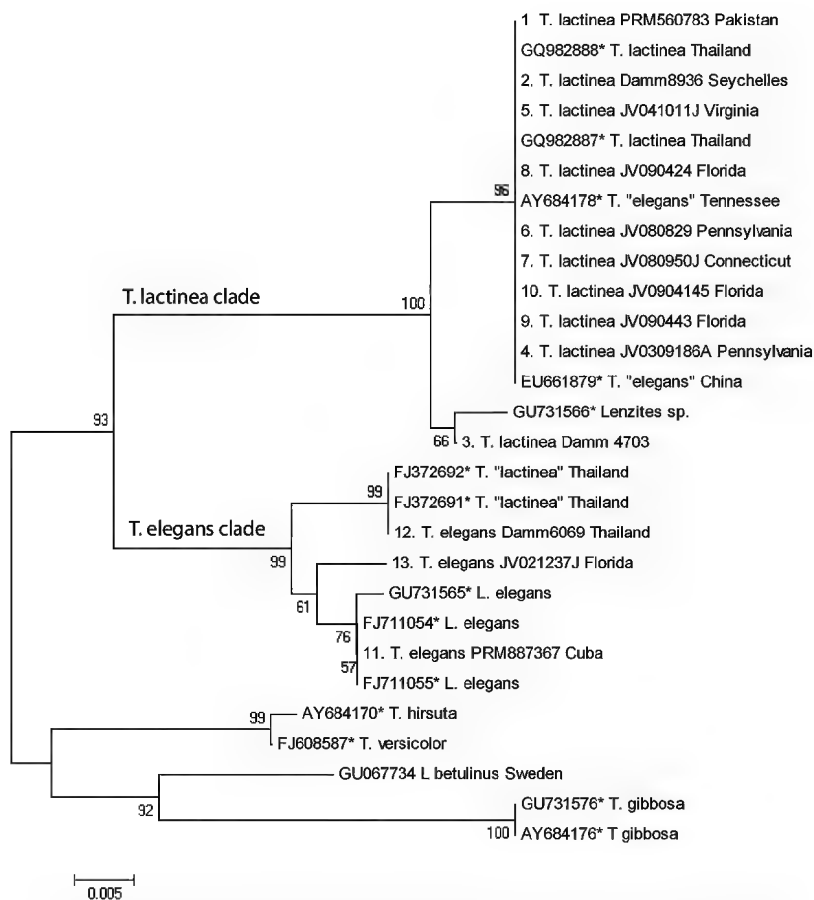


FIGURE 2: Evolutionary relationships of 28 *Trametes* species based on ITS region sequence. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in the units of the number of base substitutions per site. Accession numbers with * represent sequences retrieved from GenBank. Other GenBank accession numbers are in TABLE 1.

ITS sequence analysis

All our collections and one PRM herbarium specimen from Pakistan showed striking sequence similarities with only one variable site in ITS1. The sequence is identical with two recently published sequences (GQ982887, GQ982888) of

Trametes lactinea from Thailand and also with sequences AY684178 (Tennessee; Tomšovský et al. 2006) and EU661879 (China, unpublished) that were derived from putative *T. elegans* collections (FIG. 2). The GenBank sequence GU731566, identified as "*Lenzites* sp." and derived from the CIRM-BRFM 1050 strain and also somewhat aberrant specimen of F. Dammrich from Mauritius show about 15 transitions/transversions in ITS region, but overall sequence similarity with *T. lactinea* is prevails (see discussion). Other *T. elegans/lactinea* sequences from GenBank as well as from our own, F. Dammrich, and PRM collections are distinct and comprise a distinctly separate clade (FIG. 2) that represents the true *T. elegans*.

Discussion

The massive fruiting of a previously unrecognized, large polypore, *Trametes lactinea*, in southern Florida is very surprising. It is possible that the species has been misdetermined as *T. elegans* or *T. menziesii* and that many collections in American herbaria are in fact *T. lactinea*. We have not systematically studied its ecology, but it is worth mentioning that during September 2010 in our best localities around Miami, FL, we could not find any basidiocarps except for some disintegrated remnants. We infer that in the southernmost USA, the species develops very late in autumn, survives winter, and disintegrates in spring, which might contribute to its imperceptibility in the past. Nevertheless, the striking sequence homogeneity of all American collections of *T. lactinea* also suggests a relatively recent colonization of the American continent. We have previously described a similar case of the sudden appearance of the European *T. gibbosa* in the northern USA and Canada (Kout & Vlasák 2007); currently, *T. gibbosa* is the dominant polypore in the region, at least in Pennsylvania (Vlasák, unpublished observations 2010). Notably, *T. gibbosa* also had been misdetermined as *T. elegans*. More extensive studies are needed to confirm that *T. lactinea* is a recent invasive species in the USA. Long-term and population studies may also help explain the unexpected spread of *T. lactinea* to northern USA that has, to our knowledge, no parallel in its primary distribution area in Asia.

A somewhat aberrant specimen from Mauritius, Damm 4703, with a small pileus and slightly labyrinthine or split tubes and initially determined as *T. menziesii*, has a sequence that corresponds to most of that from *T. lactinea* but contains several mutations that are often identical with GenBank GU731566 derived from a *Lenzites* sp. collected in French Guiana. These two sequences cluster together with 66% bootstrap support (FIG. 2). The contributors of GU731566 note that several other French Guianan *Lenzites* sp. specimens with quite variable pore surfaces (poroid with 1-2 mm wide pores, or daedaloid, or lamellate) but with similar sequences were all sterile (A. Favel, INRA, Marseille, pers. comm.). Fertile basidiocarps are needed to solve this problem. *Trametes*

menziesii is a similar but variable paleotropical species with no sequence yet deposited in GenBank. According to Ryvarden & Johansen (1980), it should have rather narrow spores, only 1–2 µm wide. We have seen only one specimen with older name *Polyporus meleagris* Berk. in PRM herbarium. It was a very different fungus and so much damaged that we could not isolate DNA.

The *T. elegans* clade (FIG. 2) is much more divergent than that of *T. lactinea* and the division into two branches is well supported. Again, we have no good explanation for it at present. *Trametes elegans* is often regarded a member of genus *Lenzites* (*L. elegans* (Spreng.) Pat.) based on its sometimes lamellate hymenophore and some pointed ends of binding hyphae protruding into hymenium, reminiscent of the catahymenium characteristic of *L. betulinus* (L.) Fr. (Nuñez & Ryvarden 2001). Nevertheless, our ITS region sequence phylogram shows no similarity between *T. elegans* and *L. betulina*, and so we find the name *T. elegans* more appropriate.

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